

NATURAL TRANSMISSION OF *DIROFILARIA IMMITIS* BY *AEDES AEGYPTI*¹

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ABSTRACT. The Liverpool strain of the mosquito *Aedes aegypti* was infected with microfilariae of the canine heartworm, *Dirofilaria immitis*, and was used to transmit heartworm larvae to three dogs. Methods of confirming heartworm infection in these dogs included the modified Knott's test, a commercial enzyme-linked-immunosorbent assay (ELISA), an indirect fluorescent antibody (IFA) test, and post-mortem examination.

INTRODUCTION

Ludlam et al. (1970) listed 63 species of mosquitoes in which "complete larval development of *Dirofilaria immitis* has been reported." Since then, an additional 9 mosquito species capable of supporting larval development of *D. immitis* to the infective third larval stage within the head or proboscis have been reported (Crans and Feldlaufer 1974, Seeley and Bickley 1974, Weinmann and Garcia 1974, Bickley 1976, Christensen 1977, Mosha and Magayuka 1979, Rogers and Newson 1979, Acevedo 1982, Walters and Lavoipierre 1982). However, the capability of transmitting infective third stage larvae of *D. immitis* to the canine definitive host by mosquito bite has been demonstrated with only 7 of these 72 species (Bancroft 1904, Kume and Itagaki 1955, Newton 1956, Bemrick and Moorhouse 1968, Bickley et al. 1977, Christensen 1977, Hendrix et al. 1980).

Aedes aegypti (Linnaeus) has been one of the most extensively studied mosquito species with regard to its role in the larval development of *D. immitis* (Ludlam et al. 1970). Both wild and laboratory strains of this mosquito have been studied with diverse results regarding their suitabilities as intermediate hosts (Buxton and Mullen 1981, Tolbert and Johnson 1982, Sauerman and Nayar 1983).

Although *Ae. aegypti* has been used extensively to study the development of *D. immitis* (Kershaw et al. 1953, Taylor 1960, Singh et al. 1967, McGreevy et al. 1974, Lindemann 1977), transmission of *D. immitis* to dogs by bites from infectious *Ae. aegypti* in the laboratory has not been demonstrated (Hinman 1935).

Researchers who have utilized *Ae. aegypti* for infecting dogs with the canine heartworm have dissected infective third stage larvae from the mosquito and injected the larvae subcutane-

ously into the canine definitive host (Tulloch et al. 1970, Kotani and Powers 1982). The purpose of this experiment was to assess the ability of *Ae. aegypti* (Liverpool strain) to transmit infections of *D. immitis* to the dog.

MATERIALS AND METHODS

REARING TECHNIQUES. The Liverpool strain of *Ae. aegypti* was provided by Dr. John McCall, Athens, Georgia. Eggs oviposited on moist cotton batting were hatched in 22 × 11 × 5 cm white enamelware pans containing distilled water at a depth of 4 cm. Food for larval development was a mixture of 1 part rat chow, 1 part brewers' yeast and 1 part lactalbumin. Pupae were collected with a bulb syringe and transferred to 185 ml paper cups; the cups were then transferred to 30.5 × 30.5 × 30.5 cm mosquito holding cages (American Biological Supply, Baltimore MD, 21228) in an environmental chamber at 26°C, 80% relative humidity, and alternating 12-hour light and dark photoperiods. Emerged mosquitoes were maintained in the holding cages on a 5% sucrose solution (in a vial with a cotton plug wick).

INFECTION OF MOSQUITOES. The donor dog was a 22.7 kg, mixed-breed female exhibiting circulating microfilariae of *D. immitis* on the modified Knott's test (Newton and Wright 1956). Her circulating microfilaremia level averaged 150 microfilariae per 20 µl of blood. To encourage their feeding on the heartworm-infected donor dog, adult female mosquitoes were denied blood meals for 3 days following their emergence. Mosquitoes fed unhindered on the donor dog's hind leg for periods ranging from 30 min to 1 hr. Cages were then returned to the environmental chamber to allow the larval development of *D. immitis* to take place within the mosquitoes over the next 14 days.

TRANSMISSION STUDIES. The test group of 4 littermate dogs, three recipients (dogs A, C and D) and one control (dog B), had spent their entire lives in an indoor kennel environment (a "bioclean" area). Prior to the initiation of the study, blood samples from all dogs were examined using the modified Knott's test and were negative for microfilariae. Sera from each animal examined for antibodies against adult anti-

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gens of *D. immitis* using a commercial enzyme-linked immunosorbent assay (ELISA) (Dirotest, Mallinckrodt, Inc., Bohemia, NY) and for antibodies against microfilariae using an indirect fluorescent antibody (IFA) test (Dawe et al. 1980) were antibody-negative. The dogs were transferred to individual cages in a confinement area with a screened entryway. Outside doors were locked and vents sealed to prevent access of wild infectious mosquitoes to experimental and control animals.

Transmission studies were conducted 14 days following the mosquitoes' infectious blood meal. One limb of each recipient dog was inserted through the access sleeves of the holding cages and mosquitoes fed unhindered for periods ranging from 30 min to 1 hr. On any succeeding day following the initial 14 day developmental period, if surviving mosquitoes would feed unhindered on the recipient dogs, they were permitted to do so. Dog A was exposed to mosquitoes on 7 occasions, dog C on 4 occasions, and dog D on 3 occasions. Dog B was never exposed to infected caged mosquitoes but remained in the screened confinement area with her littermates, serving as a sentry control.

PREPATENT PERIOD. After the initial exposure period, all dogs were housed in individual cages in the mosquito free environment. Beginning 3 weeks postexposure, sera from recipient and control animals were collected and frozen at weekly intervals for later analyses using the ELISA and IFA tests. Three months following the initial exposure, whole blood was collected at weekly intervals from recipient and control animals and examined for microfilariae of *D. immitis* using the modified Knotts test.

NECROPSY PROCEDURES. All dogs were euthanized by lethal injection of sodium pentobarbital. Necropsy was performed on dog D 29 weeks following initial exposure to infectious mosquitoes, and on dogs A, B and C at 55 weeks postexposure. The hearts, pulmonary arteries, lungs and venae cava of the recipient and control dogs were examined for adult *D. immitis*. In addition, ectopic sites (brain, eye, etc.) were examined for the presence of adult *D. immitis* in the sentry control animal to ensure that aberrant adult heartworms were not present (Otto 1974).

RESULTS

All dogs remained healthy throughout the experiment. Dog D began to exhibit circulating microfilariae of *D. immitis* approximately 28 weeks following the initial exposure. Serum from this dog became positive for antibodies against adult *D. immitis* antigens (ELISA test) at

23 weeks postexposure and for anti-microfilarial antibodies (IFA test) at 26 weeks postexposure. At necropsy 6 adult (4 female and 2 male) *D. immitis* were recovered from the right ventricle and pulmonary arteries of dog D.

By 55 weeks following initial exposure dogs A, B and C had not exhibited circulating microfilariae of *D. immitis*. However, ELISA tests revealed that sera from dogs A and C became weakly positive for antibodies against adult antigens of *D. immitis* at 30 and 29 weeks, respectively, postexposure; sera from sentry control dog B remained negative on the ELISA test. Anti-microfilarial antibodies (IFA test) were not detected in these 3 dogs at any time during the study.

At necropsy dog A had 1 adult male *D. immitis* in the right ventricle while dog C had 2 adult females in the right ventricle. No adult *D. immitis* were present in the heart, pulmonary arteries, lungs, or venae cava of sentry control dog B nor were aberrant adult heartworms found in ectopic sites within this control animal.

DISCUSSION

Kartman (1957) reviewed much of the information concerning the interactions between *D. immitis* and the various mosquito species that are capable of serving as intermediate hosts. Much of the data then and since then were gathered in studies involving the intricate host-parasite relationship between *Ae. aegypti* and *D. immitis*: effect of microfilarial intake on the survival of the host (Kershaw et al. 1953), morphological changes occurring in larvae (Taylor 1960), encapsulation, host reaction to developing larval stages (Lindemann 1977), effects of temperature on the extrinsic incubation (Singh et al. 1967), and emergence of infective larvae from the mouthparts (McGreevy et al. 1974). Although a large amount of data has been gathered from epidemiological surveys (Tolbert and Johnson 1982, Sauerman and Nayar 1983), much has been learned concerning larval development within various laboratory strains of *Ae. aegypti*. These different strains differ markedly with regard to their host efficiency, i.e. susceptibility versus refractoriness (Buxton and Mullen 1981). Due to these widespread differences, *Ae. aegypti* has been utilized to study the genetic bases of susceptibility to infections with *D. immitis* (Kartman 1953, Sulaiman and Townson 1980).

Aedes aegypti has long been recognized as a model for developmental studies, namely in supporting larval development of *D. immitis* to the infective third larval stage. These larvae are

usually dissected from mosquitoes into various media (e.g., Ringer's solution, Hanks' balanced salt solution or NCTC 109) and the released infective larvae then collected and injected subcutaneously into recipient dogs. This was the developmental role that *Ae. aegypti* played in the past, a role of artificial transfer (Tulloch et al. 1970, Kotani and Powers 1982). Until this report, this species had never been shown to transmit the parasite successfully to the dog (Hinman 1935).

The maturation of larvae to the infective third larval stage within the proboscis does not guarantee the capacity of a mosquito species to transmit the parasite to the dog (Crans and Feldlaufer 1974, Seeley and Bickley 1974, Bickley 1976). In Hinman's study (1935) with an unknown strain of *Ae. aegypti*, the author states that the "maturation of the larvae within the insect host and its migration to the proboscis has always been assumed to be *prima facie* evidence of ability to transmit the given filarial infection. However, it is entirely possible that many blood-sucking insects, discovered to be susceptible to experimental infection and even found naturally infected, are unable to successfully transmit the parasite. Practically all of our knowledge of transmission of filarial organisms rests upon such circumstantial evidence, which may be quite fallacious."

In the present study, very few adult *D. immitis* were transferred to the dogs by bites from *Ae. aegypti*. Two of 3 recipient dogs in the present study were amicrofilaremic because they harbored single sex infections. This type of infection is impossible to diagnose using the modified Knott's technique and the IFA test as microfilariae are not produced in single sex infections. Single sex infections, however, may be detected in dogs by using the ELISA test which detects antibodies against antigens of adult *D. immitis*.

This study has demonstrated that the bite of *Ae. aegypti* (Liverpool strain) may be used to transmit *D. immitis* from mosquito to dog. The primary role of *Ae. aegypti* in transmission studies, however, should remain limited to the production of infective third stage larvae for use in inoculative transfer studies. These inoculative methods tend to produce greater numbers of adult worms in the right ventricle and pulmonary arteries and are thus a more efficient utilization of *Ae. aegypti* (Liverpool strain) as an intermediate host of *D. immitis*.

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